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Prostaglandin E_1 action on sinus pacemaker and adenylyl cyclase in kitten myocardium

A QUANTUM mechanical analysis of Kier *et al.*[1,2] indicates that the amine group of the catecholamine isoprenaline may not be essential to produce pharmacological effects mediated through adrenergic β receptors. Based on this and on conformational similarities between the ring, β-hydroxyl group and alkyl areas of N-*n*-propylnoradrenaline and prostaglandin (PG) E_1, these authors predicted that PGE_1 should be able to interact positively with adrenergic β receptors. Isolated tissues from kitten heart are appropriate systems to investigate effects of drugs which are β-receptor dependent and we tested the predictions of Kier *et al.* by investigating if PGE_1 produced positive inotropic and chronotropic effects and whether these effects are transmitted through the adrenergic β receptors.

It is a general finding that dose response curves of positive inotropic and chronotropic effects of catecholamines are similar when obtained with driven left atrial strips and with spontaneously beating right atria[3]. We found that PGE_1 selectively produced positive chronotropic effects but not positive inotropic effects (Fig. 1). Similarly, on six driven papillary muscles (5 s intervals, 32.5° C, from kittens pretreated with reserpine), concentrations from 2×10^{-8} M to 4×10^{-5} M PGE_1 did not modify the strength of isometric contraction or the rates of contractions and relaxation. This dissociation of positive chronotropic effects and lack of inotropic effects of PGE_1 is not characteristic for adrenergic agonists.

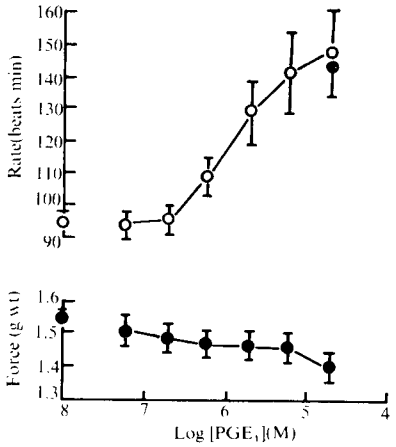

Fig. 1 Nonadrenergic positive chronotropic effect of prostaglandin PGE_1. Tissues were from kittens (weight 350–800 g, either sex), pretreated with reserpine (5 mg kg^{-1} subcutaneously, 24 h). Cumulative concentration effect curves of PGE_1 on spontaneously beating right atria (○) and on left atrial strips driven at 2 s intervals with barely suprathreshold pulses of 5 ms duration (●). Values are mean ± s.e.m. from four pairs of left and right atria in the same 50 ml organ bath containing gassed (95% O_2, 5% CO_2), warm (32.5° C) modified Krebs solution[3]. 6×10^{-7} M (±)-propranolol was added for 65 min after 2×10^{-5} M PGE_1 had produced an equilibrium effect on right atria. (±)-Propranolol did not influence the effect of PGE_1 (cross in circle). Open and closed circles on ordinate are values of basal rate and force, respectively, before PGE_1 was administered.

To see whether the selective positive chronotropic effect of PGE_1 on right atria was mediated by adrenergic β receptors, β-receptor blocking agents were used. Thus, if PGE_1 is a β agonist its activity should be antagonised by conventional β-blockers and greater concentrations of the prostaglandin should surmount the blockade allowing the estimation of an apparent dissociation equilibrium constant (K_B) for the blocker–receptor interaction. If the blocker and the prostaglandin interact both with the same receptor this K_B should not differ from the K_B estimated from the antagonism of the blocker against a classical β agonist such as isoprenaline. With this in mind, we first added 6×10^{-7}M (±)-propranolol to the atria under the influence of PGE_1. (±)-Propranolol did not modify the positive chronotropic effect of PGE_1 on four right atria (Fig. 1). In two of these atria 1×10^{-7}M (−)-KL 255 was administered 65 min after (±)-propranolol and an additional 30 min incubation of (±)-propranolol plus KL 255 did not influence the positive chronotropic effect of PGE_1. Because the employed concentrations of both (±)-propranolol and (−)-KL 255 were at least 200 times greater than their respective K_Bs[3,4] they produced at least 99.5% β-receptor occupancy. Since the positive chronotropic effect of PGE_1 persisted under these conditions, it must be ruled out that it is mediated through adrenergic β receptors[1,2,5].

PGE_1 presumably modifies the ion permeability of the membrane of sinus pacemaker cells in such a way that steeper prepotentials and tachycardia result. If so, it may perhaps be related to an increase of adenylyl cyclase activity in the membrane of pacemaker cells and concomitant increase of intracellular cyclic 3′,5′-adenosine monophosphate (cyclic AMP) levels. Based on the previous findings, activation of pacemaker cell adenylyl cyclase by PGE_1 should be independent of β-receptor action. We therefore explored the possible effect of PGE_1 and isoprenaline on adenylyl cyclase activity in membranes of kitten atria. In such membranes PGE_1 increases adenylyl cyclase activity by 50% (Fig. 2). As expected, this effect of PGE_1 is unaffected by 10^{-7}M (±)-propranolol, indicating that it is not mediated by adrenergic β receptors. To

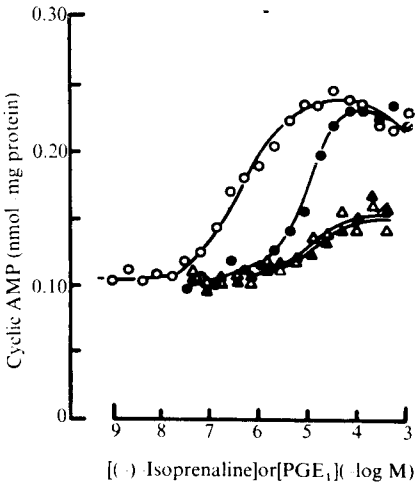

Fig. 2 Nonadrenergic and adrenergic adenylyl cyclase stimulation by PGE_1 and (−)-isoprenaline, respectively. 10,000*g* membrane particles of atria from kittens pretreated with reserpine were prepared as described elsewhere[12]. Particles (78 μg protein) were preincubated in 20 μl of 0.5 mM $KHCO_3$, 2.5 mM EGTA, 25 mM Tris-HCl, *p*H 7.5, at 4° C, during 120 min with (solid symbols) or without (open symbols) 10^{-7} M (±)-propranolol; later 10 μl of 1 mM $KHCO_3$, 0.05 mM EGTA, *p*H 7.5, with or without (−)-isoprenaline (circles) or PGE_1 (triangles) were added and the mixture further preincubated for 90 min at 4° C. Incubation was initiated by adding 20 μl containing ingredients to give in a final volume of 50 μl: 2.0 mM α-^{32}P-ATP (1.5×10^6 c.p.m.), 3.5 mM $MgCl_2$, 1.0 mM cyclic AMP, 20 mM creatine phosphate, 0.2 mg ml^{-1} creatine kinase and 25 mM Tris-HCl, *p*H 7.5. The incubation lasted 10 min at 32.5° C; it was stopped with 100 μl of 40 mM unlabelled ATP, 12.5 mM ^{3}H-cyclic AMP (about 10,000 c.p.m.) and 1% sodium dodecyl sulphate followed by immediate boiling for 3.5 min. The ^{32}P-cyclic AMP formed was determined by the method of Krishna *et al.*[13].

check whether these membranes preserved their β receptors, the action of (−)-isoprenaline was investigated. (−)-Isoprenaline increased adenylyl cyclase activity about three times more than PGE_1. (−)-Propranolol (10^{-7}M) surmountably antagonised the action of (−)-isoprenaline (Fig. 2).

We calculated an apparent K_B of about 3×10^{-9}M for (±)-propranolol from the concentration ratio of concentration-effect curves of (−)-isoprenaline obtained in the absence and the presence of the blocker according to equation K_B = (±)-propranolol/(concentration ratio — 1). This value of K_B for (±)-propranolol is the same as the K_B between the β blocker and receptors mediating positive inotropic and chronotropic responses of (−)-isoprenalaine[4], indicating that the β receptors are unchanged in cell-free membrane particles[6].

Concentration effect curves to PGE_1 on both intact right atria—positive chronotropic effect, and on atrial membrane particles—stimulation of adenylyl cyclase, seem to correlate. This correlation does not however necessarily imply functional dependency. If the enhanced adenylyl cyclase activity is a specific step between a hypothetical PGE_1 receptor and a site on the pacemaker cell where permeability changes lead to increased chronotropism, one would expect adenylyl cyclase in membrane particles from the ventricle not to respond to PGE_1. But PGE_1 was found to increase also the adenylyl cyclase activity in membranes from ventricular myocardium (Fig. 3). This effect is only about 2/5 of the maximum increase of activity obtained with (−)-isoprenaline. As in atrial membranes, the effect of PGE_1 is not modified by 1×10^{-7}M (±)-propranolol and seems therefore not to be mediated by adrenergic β receptors. In contrast, (±)-propranolol surmountably shifted the concentration-effect curve of (−)-isoprenaline towards greater amine concentrations (Fig. 3) which allowed us to estimate a K_B of about 4×10^{-9}M for (±)-propranolol. This K_B is the same as the K_B between (±)-propranolol and β receptors mediating inotropic effects in papillary muscles of kittens, indicating that the β receptors are preserved in ventricular membrane particles[6]. The similarity of K_Bs of (±)-propranolol on membrane particles from atria and ventricles is evidence that the membranes from both tissues have similar β receptors.

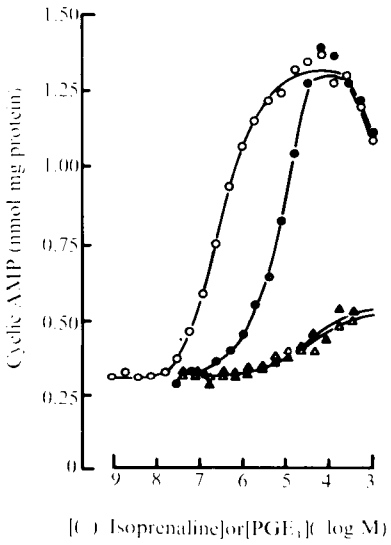

Fig. 3 Comparison of adenylyl cyclase stimulation by PGE_1 (triangles) and by (−)-isoprenaline (circles) on membrane particles of ventricle. For incubation medium and other conditions, see legend to Fig. 2. Solid symbols: with 10^{-7} M (±)-propranolol; open symbols: without (±)-propranolol.

Our results support the suggestion that PGE_1 would increase chronotropism through an increase of adenylyl cyclase activity in cardiac membranes. Cyclic AMP has been shown to increase activity of pacemaker cells not only in atria, but also in Purkinje fibres. The increase of heart cyclic AMP[8], and membrane adenylyl cyclase activity to PGE_1 seems to be of no consequence for inotropism and if PGE_1 promotes chronotropism through stimulation of adenylate cyclase activity, this effect is either restricted to intact pacemaker cells or does not affect mechanisms that lead to increased strength of contraction of myocardium. The fact that PGE_1 stimulates adenylyl cyclase and chronotropism through receptors which are not adrenergic β receptors does not rule out the possibility that catecholamines perhaps catalyse the synthesis of endogenous prostaglandins. This has been suggested for the action of cyclase stimulating hormones in the ovary[9], the thyroid[10], and stomach muscle [11].

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A. J. KAUMANN

Centro de Investigaciones Cardiologicas,
M.T. de Alvear 2270,
Buenos Aires, Argentina

LUTZ BIRNBAUMER

Department of Physiology,
Northwestern University Medical Center,
Chicago, Illinois 60611

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